Src and podoplanin forge a path to destruction

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Cancer and arthritis present an enormous challenge to society. They share pathogenic pathways that involve extracellular matrix degradation, tissue invasion, and inflammation. Most cancer and arthritis treatments affect normal cell function to cause significant adverse effects in patients. Specific pathways that promote cancer and arthritis progression must be elucidated to design more targeted and effective therapeutics. The Src kinase and podoplanin (PDPN) receptor are upregulated in cancer cells, fibroblasts, synoviocytes, and immune cells that increase tissue invasion and inflammation to promote both cancer and arthritis. In this review, we discuss how Src and PDPN forge a path to tissue destruction, and how they can serve as targets for therapeutics to combat cancer and arthritis.

Cancer and arthritis

Cancer and arthritis have reached epidemic proportions. Cancer causes over 10% of all deaths worldwide, killing over 8 million people around the world every year, which is >15 people every minute [1]. Transformation of normal cells to cancer cells results from changes in cellular signaling pathways that otherwise regulate cell growth and motility [2].

While uncontrolled growth and unchecked migration of cancer cells have a significant part in cancer progression, signals from the tumor microenvironment can further aggravate the disease. Cancer cells, cancer-associated fibroblasts (CAFs), and immune cells secrete cytokines, growth factors, and metalloproteases that degrade the extracellular matrix (ECM) to facilitate cancer growth, invasion, and metastasis (outlined in Fig. 1) [2–6]. This process involves a host of proinflammatory cytokines and pathways similar to wound healing and inflammation programs [7–9].

As with cancer, arthritis also induces ECM degradation by proteolytic enzymes. This can destroy the cartilage between bones to cause joint swelling, stiffness, deformity, severe pain, and decreased mobility in patients [10,11]. Over 10% of men and 18% of women over 60-years old have osteoarthritis (OA) caused by destruction of joint cartilage and underlying bone [12]. OA pathophysiology is characterized by progressive loss of articular cartilage, subchondral bone thickening and sclerosis, new bone formation at the joint margins, and increased vascularization of calcified cartilage, which expands into, and invades, the articular cartilage (Fig. 2). Although OA was initially classified as noninflammatory arthritis, there is increasing evidence that joint inflammation is a key factor in OA pathogenesis [13,14].

In addition to OA, rheumatoid arthritis (RA) has an inflammatory origin and is found in ~1% of the world population between 20 and 40 years of age [15]. RA is characterized by inflammation of the synovium (connective tissue lining the joints), bone, and articular cartilage destruction (Fig. 2). The synovium becomes infiltrated with lymphocytes during RA pathogenesis. These lymphocytes produce inflammatory cytokines and autoantibodies that increase vascularization of the synovial membrane. Cells in the synovial membrane, namely macrophage-like cells (MLCs) and fibroblast-like synoviocytes (FLSs), proliferate and recruit osteoclasts to form a hyperplastic 'pannus' that covers the articular cartilage. Cells in pannus tissue secrete proinflammatory cytokines and matrix metalloproteases, which invade and erode surrounding cartilage and bone by mechanisms similar to tumor invasion (Figs. 2 and 3C) [16–18].

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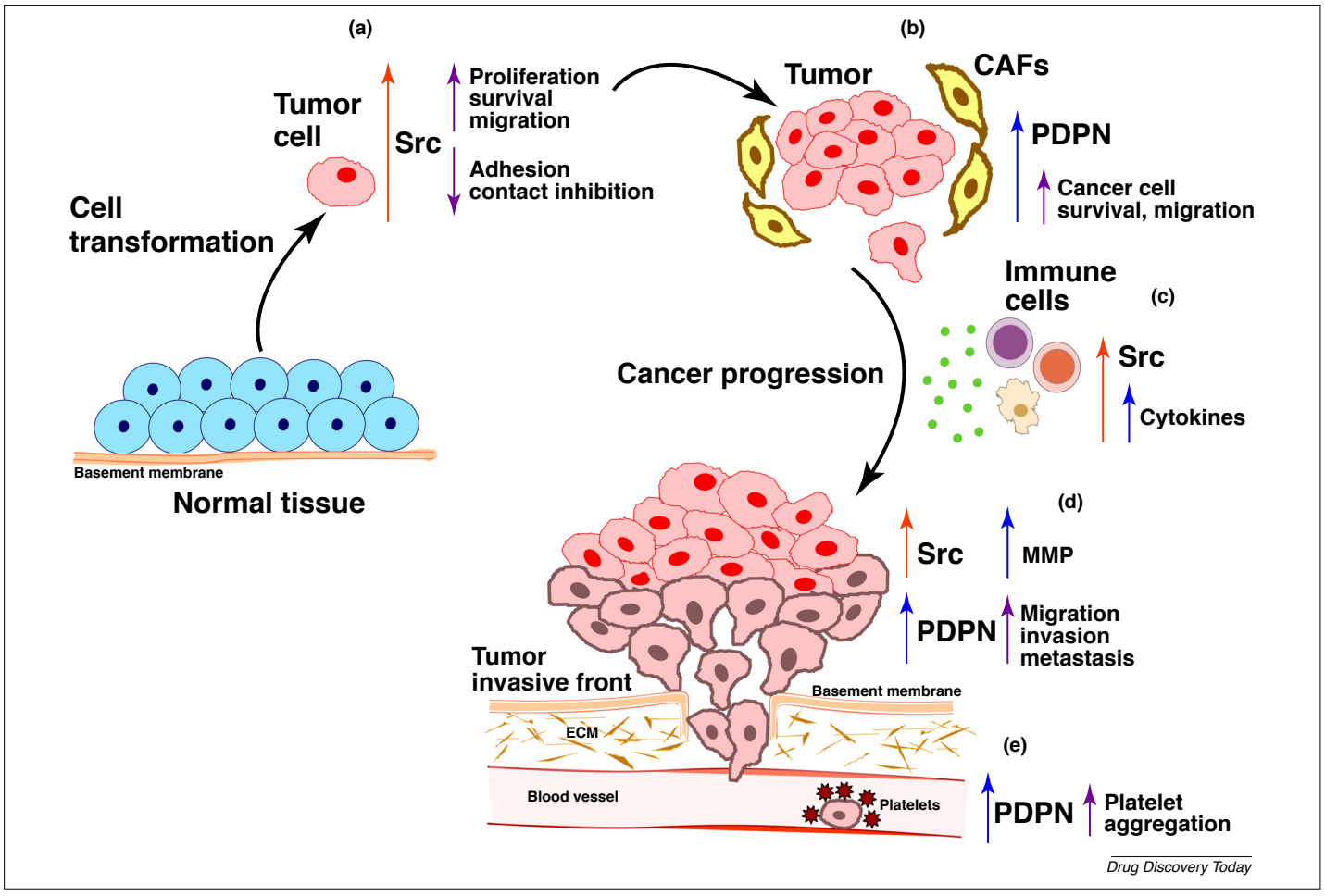

FIGURE 1

Role of Src and podoplanin (PDPN) in cancer progression. (a) Increased activation of the oncogenic tyrosine kinase Src increases transformed cell growth, survival, and migration, and decreases cell adhesion and cell contact inhibition to promote tumor progression. (b) Tumor cells interact with cancer-associated fibroblasts (CAFs) in the tumor microenvironment. Elevated expression of the glycoprotein receptor PDPN in CAFs has been shown to promote cancer cell survival, migration, and metastasis. (c) Cancer cells can also recruit immune cells, such as macrophages and monocytes, to the tumor microenvironment. Activation of Src kinase in these immune cells increases the production of specific inflammatory cytokines that contribute to cancer progression. (d) Src kinase activity also induces PDPN expression to augment tumor cell migration and invasion. PDPN expression is particularly abundant at the invasive front of tumors. Src kinase activity and PDPN expression in tumors correlates with matrix metalloproteases (MMPs), which degrade the extracellular matrix (ECM) to facilitate tumor cell invasion. (e) In blood vessels, PDPN in tumor cells interacts with C-type lectin 2 (CLEC-2) on platelets to induce platelet aggregation around the tumor cells, which induces hematogenous metastasis. Activation is indicated by red arrows, increased expression is indicated by blue arrows, and changes in cellular processes by purple arrows.

Routine options for arthritis treatment include disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate, steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and biologics [19,20]. Biologics primarily target TNFα, JAK kinase, cytokines or their receptors, which participate in the inflammatory response [19]. However, these biologics interfere with normal immune responses, causing patients to become susceptible to infections and malignancies [21].

Interestingly, some treatments for arthritis are also utilized for cancer therapy. For example, NSAIDs are commonly used to alleviate inflammation and pain in patients with arthritis. NSAIDs act by inhibiting cyclo-oxygenases (COX-1 and COX-2) that produce inflammatory prostaglandins [22], and can reduce risk and tumor burden from breast, colorectal, esophageal, and gastric carcinomas [23–25]. However, NSAIDs have adverse effects on kidneys, liver, and cardiovascular system [22,25]. More selective NSAIDs, which target COX-2, such as rofecoxib and celecoxib, can also be used to treat OA and RA [26]. However, rofecoxib presents significant adverse effects and safety concerns. Celecoxib is still prescribed for arthritis, but can cause cardiovascular and upper gastrointestinal complications [27]. Celecoxib is also used as a chemopreventive for patients with colorectal cancer and familial adenomatous polyposis coli [28,29].

It is clear that inflammation and tissue degradation are common to both cancer and arthritis pathophysiology [25]. However, mechanistic links between these two major maladies have not yet been defined. Fundamental aspects of these links stem from the Src tyrosine kinase and podoplanin (PDPN) receptor.

Src kinase in cancer and arthritis

The Src tyrosine kinase is anchored to the cytoplasmic side of the cell membrane by an N-terminal myristoylation site, followed by SH3 and SH2 domains that mediate protein interactions, and a kinase domain that contains positive and negative regulatory

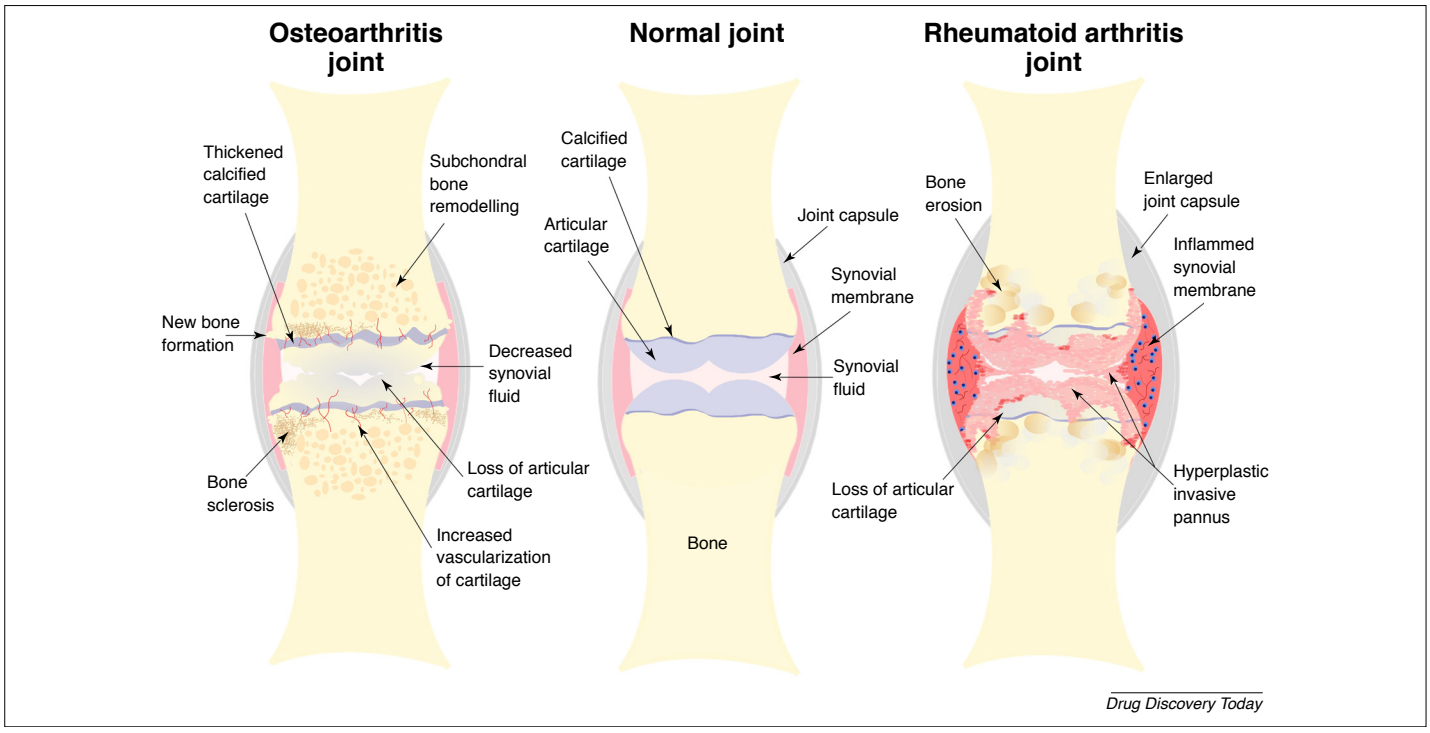

FIGURE 2

Schematic of normal, osteoarthritis, and rheumatoid arthritis joints. Healthy joints (center) comprise articulating bones that are held together by ligaments that form a capsule. The synovial membrane forms the inner layer of the joint capsule and secretes synovial fluid that lubricates the joint surfaces. The joint surface is lined by articular cartilage comprising extracellular matrix material and chondrocytes. The articular cartilage provides smooth joint movement and resistance to mechanical forces. The layer of bone beneath the cartilage is the subchondral bone. Calcified cartilage secures subchondral bones to the articular cartilage. Osteoarthritis (OA) is caused by mechanical wear and tear of the joints because of aging or injury. OA is characterized by progressive loss of articular cartilage accompanied by decreased synovial fluid, new bone formation at the margins, subchondral bone sclerosis, subchondral bone remodeling, and increased vascularization of the cartilage (left). By contrast, rheumatoid arthritis is characterized by inflammation of the synovial membrane and enlargement of the joint capsule. The cells of the synovial membrane recruit osteoclasts to form a hyperplastic, invasive 'pannus' tissue that eventually erodes the articular cartilage and bone (right).

phosphorylation residues [30,31]. Src phosphorylates numerous substrates, including p130Cas, catenins, phosphatases, and other proteins that control cell morphology and behavior [30,31]. Src also activates other kinases, including FAK, MAPK, and Akt, as well as Rho GTPases, to reorganize the actin cytoskeleton and promote cell growth, survival, and motility [32,33].

Increased Src activity is found in many malignancies, including colorectal, breast, and pancreatic cancer [2,34,35]. Src increases invadopodia formation and matrix metalloprotease (MMPs) production (e.g., MMP-2 and MMP-9) that degrade the ECM during tumor invasion (Fig. 1) [36,37]. Similar to tumor cells, Src also induces arthritic FLSs to form invadopodia that secrete MMPs (e.g., MMP-3 and MMP-13) to degrade the ECM in the articular cartilage (Fig. 3) [38,39]. Src and FAK also promote MLC and FLS migration into arthritic synovial tissues [40].

Src and other members of the Src kinase family activate pathways that produce cytokines to augment inflammatory responses [41–43]. For example, TNFα stimulates Src to activate the nuclear factor (NF)-κB transcription factor in macrophages, where it promotes the transcription of inflammatory cytokines, such as IL-6 [44,45]. Src family kinases also interact with the IL-6 receptor signaling protein gp-130 to potentiate IL-6 signaling [42]. Moreover, Src acts downstream of macrophage colony-stimulating factor receptors to activate the PI-3K pathway [46].

Inflammatory cytokines have an important role in arthritis progression. TNFα and interleukins, such as IL-1β and IL-18, increase Src phosphorylation in RA FLSs and MLCs [40,47]. Activation of Src further stimulates PI3K/Akt and MAPK/ERK pathways, which can increase FLS VCAM1 expression (Fig. 3) [47]. VCAM1 partners with integrins to recruit lymphocytes during arthritis progression [48].

In addition to inflammatory cytokines, Src is activated by integrin signaling to regulate osteoclast (macrophage-like cells in the bone) function [49]. The receptor activator of NF-κB ligand (RANKL) and its receptor (RANK) activate Src and subsequently Akt to promote bone loss in arthritis by increasing osteoclast genesis and activity (Fig. 3) [49,50]. In general, Src promotes osteoclast function of regulating bone resorption [51], and leukocyte rolling and migration in response to selectins [31,42].

Clearly, Src affects many pathways to augment immune cell recruitment and activation. Accordingly, Src kinase blockers inhibit arthritis and cancer progression [52–54]. Dual kinase inhibitors that block Src and Abl kinase activity, such as dasatinib and bosutinib, are used to treat chronic myeloid leukemia and acute lymphocytic leukemia [30,55]. More specific Src inhibitors, including MC-25, inhibit breast cancer cell migration [56]. Other tyrosine kinase inhibitors, including the SYK blocker fostamatinib and the JAK blockers tofacitinib and baricitinib, are used to treat RA [57]. However, Src kinase activity is ubiquitous and inhibition causes unacceptable adverse effects and drug resistance [54,58,59].

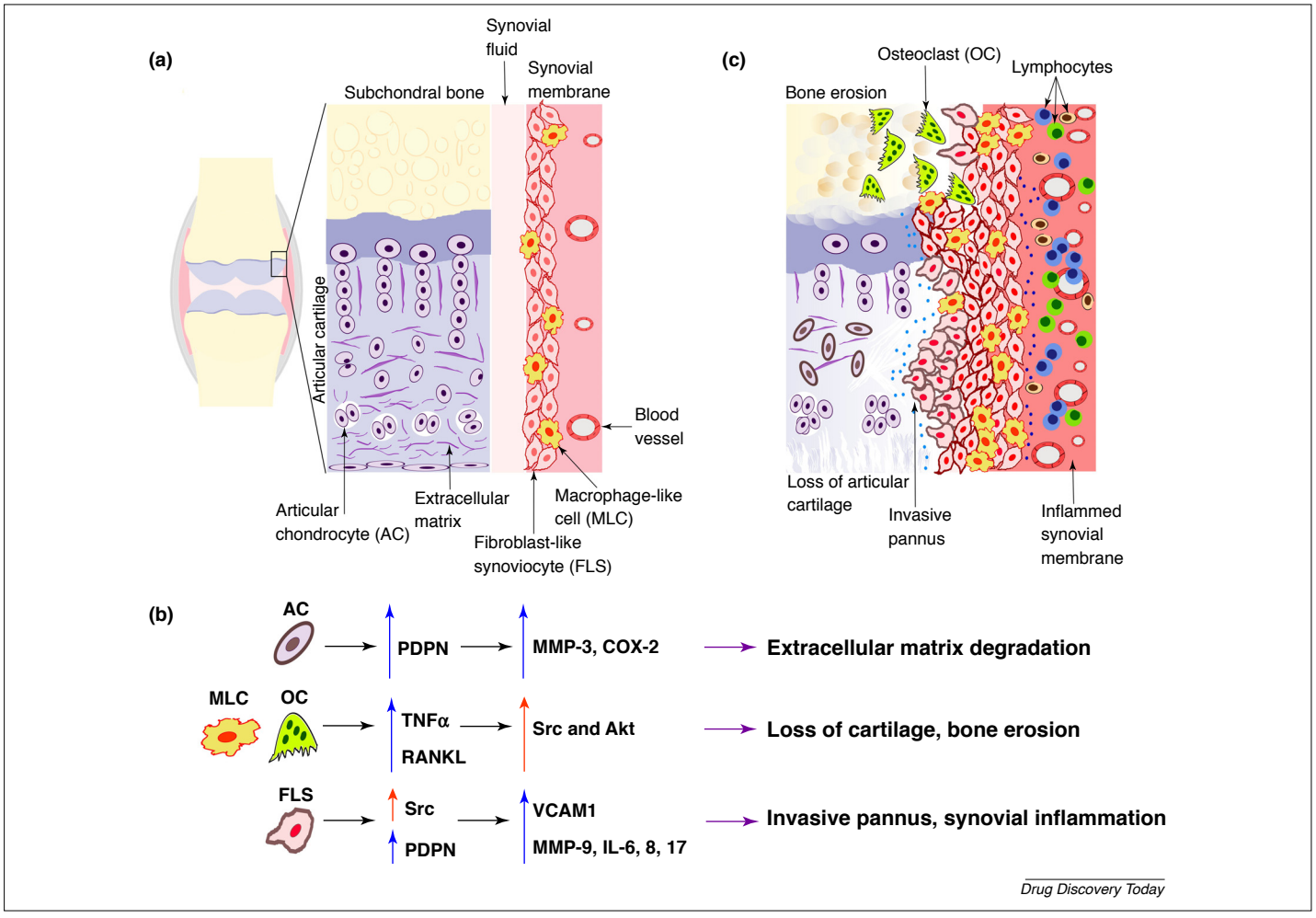

FIGURE 3

Role of Src and podoplanin (PDPN) in arthritis progression. (a) The articular cartilage comprises articular chondrocytes (AC) distributed in various zones and dense extracellular matrix (ECM). Space between articular joints is filled with synovial fluid secreted by cells of the synovial membrane. The synovial membrane comprises fibroblast-like synoviocytes (FLSs) and macrophage-like cells (MLCs). (b) Src kinase and the glycoprotein receptor PDPN promote arthritis progression. Increased PDPN expression in AC is correlated with increased with matrix metalloprotease (MMP)and cyclo-oxygenase 2 (COX-2) expression, which promotes cartilage degradation [111]. Increased expression of cytokines, including TNFα and receptor activator of NF-κB ligand (RANKL), activate Src kinase in MLCs and osteoclasts (OC) to promote cartilage and bone erosion, respectively. Increased Src kinase activity and PDPN expression in FLSs activate VCAM1, MMPs, and interleukins (IL), which augments 'pannus' tissue invasion and synovial inflammation. (c) Inflammation is a characteristic feature of RA. The synovium becomes infiltrated with lymphocytes during RA pathogenesis. These lymphocytes produce inflammatory cytokines that increase vascularization of the synovial membrane. MLCs and FLSs in the synovial membrane proliferate and recruit osteoclasts (OC) to form a hyperplastic 'pannus' that erodes the articular cartilage and bone. FLSs secrete proinflammatory cytokines and MMPs, which causes them to act like an invasive tumor and degrade extracellular matrix in the cartilage. Indeed, activation of Src and PDPN in various cells of the joint tissue promotes bone and cartilage erosion, hyperplastic pannus invasion, and inflammation.

Downstream effectors of Src are more specific and enticing targets for therapeutics.

Podoplanin in cancer and arthritis

Src phosphorylates several effectors that control cell survival, anchorage independence, differentiation, morphology, growth, and motility. Effectors that specifically promote tumor progression and inflammation could serve as operative targets to combat cancer and arthritis while minimizing adverse effects that result from upstream kinase blockers. PDPN has emerged as a powerful Src effector and therapeutic target [60,61].

PDPN is a type I receptor with a highly *O*-glycosylated extracellular region of ~130 amino acids, a single-pass transmembrane domain, and a short intracellular sequence of approximately ten amino acids. The extracellular region contains four platelet-aggregating (PLAG) domain repeats that can bind to the C-type lectin 2 (CLEC-2) receptor on platelets to induce aggregation. The intracellular sequence contains basic amino acids and serine residues, which associate with ezrin-radixin-moesin (ERM) proteins that direct RhoA GTPases to reorganize actin cytoskeleton to promote cell motility [62–64].

Src phosphorylates the Cas adaptor protein to induce PDPN expression to promote cell motility [60,65], as well as the ability of tumor cells to escape contact normalization exerted on them by surrounding nontransformed cells [2,66]. PDPN is found at the invasive front of tumors, where it promotes the progression of many types of cancer, including mammary carcinoma [64,67,68], glioma [69–72], melanoma [73–77], mesothelioma [78–80], and oral squamous cell carcinoma [61,81–84] (reviewed in [2,61,83,85–89]). Similar to Src, PDPN localizes to invadopodia to facilitate

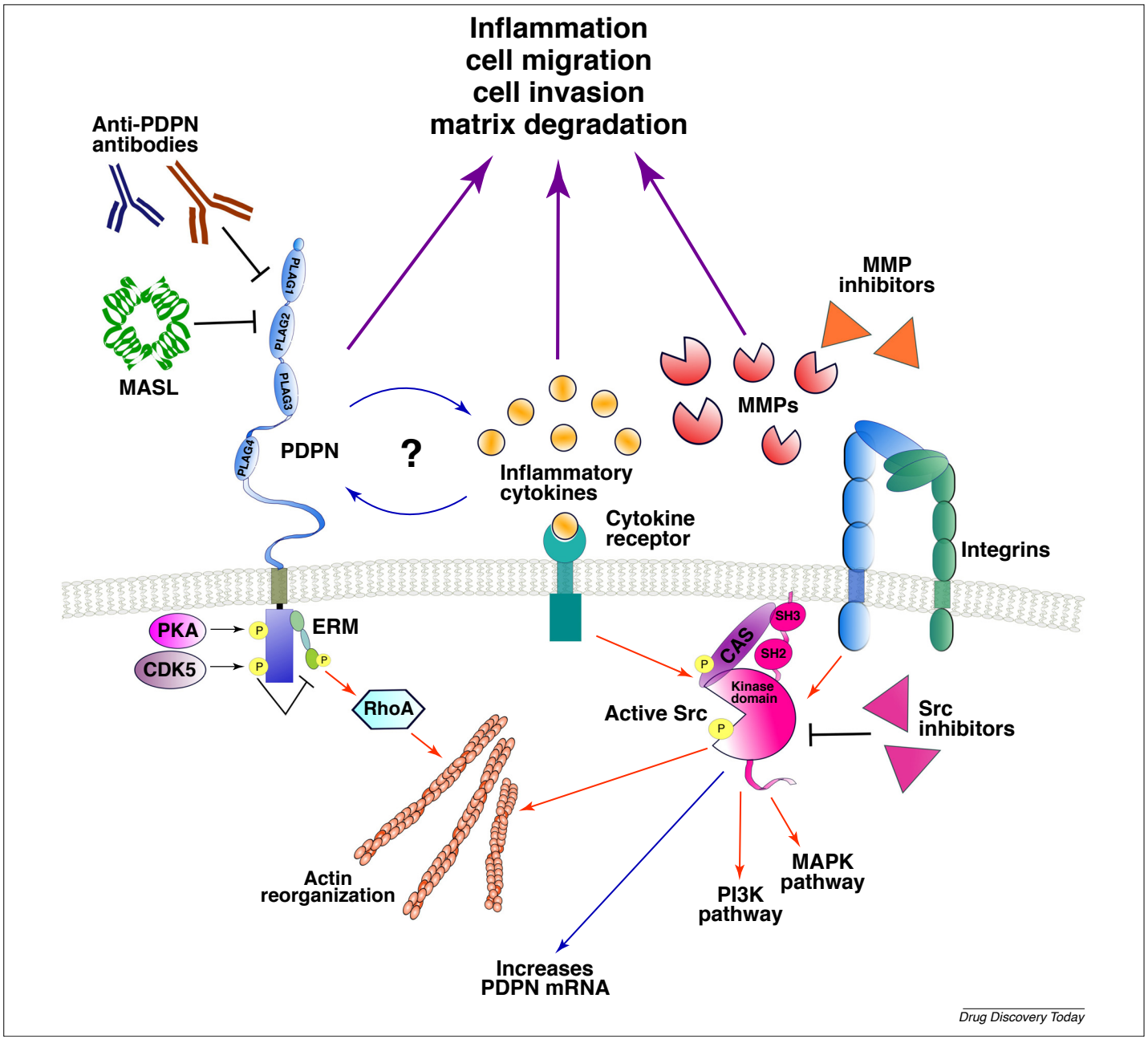

FIGURE 4

Src and podoplanin (PDPN) forge a path to destruction, and ways to block it. PDPN is a transmembrane glycoprotein with a large extracellular domain containing four platelet-aggregating domains (PLAG), and a short intracellular domain. The latter has a cluster of basic amino acids that bind to ERM family proteins to activate Rho GTPases, which helps reorganize actin cytoskeleton and promote cell migration and invasion. PDPN intracellular serine residues can be phosphorylated by PKA and CDK5 to inhibit PDPN-mediated cell migration. PDPN and inflammatory cytokines stimulate the expression of the other by unknown mechanisms, leading to increased inflammation. Src kinase is activated in response to inflammatory cytokines and integrin signaling. Activated Src phosphorylates CAS to activate PI3K and MAPK pathways, induce PDPN mRNA transcription, and trigger pathways that promote actin reorganization. Furthermore, Src kinase activation and PDPN expression lead to increased matrix metalloprotease (MMP) expression and matrix degradation. Thus, Src kinase and PDPN work together to activate destructive pathways that lead to inflammation, cell migration, cell invasion, and matrix degradation common to both cancer and arthritis. This destructive relationship can be inhibited by antibodies and other reagents (exemplified by *Maackia amurensis* seed lectin; MASL) that target PDPN to inhibit cell migration, invasion, and possibly inflammation. Src kinase activity can also be suppressed by specific blockers, whereas MMP inhibitors can block matrix degradation. Efforts are warranted to investigate how these agents could be used in combination to combat cancer and arthritis.

tumor cell invasion [90,91] (Fig. 1). PDPN expression has been shown to augment MMP expression, which degrades the ECM during tumor cell invasion [64,92–94].

In addition to cancer cells, PDPN is also expressed by cancer-associated fibroblasts (CAFs) in tumors such as gastric cancer [95], lung adenocarcinoma [96], oral cancer [97], melanoma [76], breast cancer [98,99], pancreatic ductal carcinoma [100], among others (reviewed in [88,101,102]), where it is associated with decreased survival and poor cancer prognosis. In these cancers, and as illustrated in Fig. 1, PDPN motivates CAFs to disrupt and remodel

the tumor microenvironment to facilitate cancer growth, migration, and metastasis [76,100,103–105]. The tumor cells follow paths forged by CAFs to colonize new areas and metastasize [3].

PDPN promotes inflammation and invasion associated with arthritis [106–111]. For example, during RA pathogenesis, PDPN-expressing Th17 helper T cells infiltrate inflamed joint tissues [107]. PDPN is also expressed in the FLSs of patients with RA and appears during early stages of the disease [106,110,112]. PDPN expression is particularly strong in areas of hyperplasia and disrupted tissue architecture [106], and is seen in synovia and chondrocytes in patients with OA (Fig. 3) [109,111].

As described above, PDPN expressed on tumor cells binds with CLEC-2 on platelets to induce platelet aggregation, which can promote tumor metastasis [113–116]. Indeed, agents that block PDPN-CLEC-2 interactions inhibit platelet aggregation and decrease tumor metastasis [115,117,118]. CLEC-2 expression is maintained in the peripheral blood and synovial tissue platelets of patients with RA [108,119]. In fact, platelet co-cultures with synovial fibroblasts expressing PDPN induce the expression of proinflammatory cytokines, including IL-6, IL-8, CXCL2, and CXCL3 mRNA, indicating that platelets exert a proinflammatory effect on synovial fibroblasts [108,120]. Therefore, while studies indicate that PDPN–CLEC-2 interactions can ameliorate the intensity of inflammatory reactions that produce septic shock [121], PDPN offers a target that can be used to inhibit cancer progression and arthritic inflammation.

Inflammatory cytokines induce PDPN expression by a feedback loop in which PDPN and cytokines induce the expression of the other to the detriment of the tissue architecture. CD45-positive inflammatory cells induce the expression of interferon-responsive genes in squamous cell carcinoma cells that express PDPN at invasive fronts. In return, interferon γ, TGFβ, and TNFα induce PDPN expression at the invasive edges of the tumor [122].

Arthritic joints are also rich in inflammatory cytokines. PDPN expression is upregulated by cytokines including IL-1β and TNFα [106], and PDPN expression can itself induce the expression of cytokines in RA synoviocytes, including IL-6, IL-8, and IL-17 (Fig. 3) [108,123]. Cytokines are valid targets for therapeutics to treat cancer and arthritis. Biologic DMARDs target cytokines, such as TNFα. However, these reagents are associated with increased risk of infections and malignancies because of suppression of immune surveillance [21].

In addition to cytokines, PDPN augments MMP expression to promote arthritic progression (Fig. 3) [106,111]. PDPN expression correlates with high MMP-9 expression in FLSs to promote tissue invasion in a manner reminiscent of tumor invasion [106]. There is evidence that activated platelets can be a source of MMPs, including MMP-1, MMP-2, and MMP-9 [124,125], and that these might promote cancer metastasis [126] and arthritic tissue destruction [125,127]. However, the relative contribution of MMPs from platelets, compared with actual tumor cells and associated fibroblasts, has not been clearly defined for cancer or arthritis.

MMP blockers have shown promising results in animal models of arthritis. However, they fail to show clinical benefit in human clinical trials. MMP inhibitors, including batimastat, marimastat, and tanomastat, present problems with solubility, bioavailability, toxicities, and lack of efficacy in patients with cancer [128,129].

Inflammatory cytokines and MMPs have widespread expression in the body and are required for normal physiology. Therefore, inhibition of cytokines and MMPs leads to significant adverse effects and complications that outweigh the benefits of treatment. PDPN has emerged as a pivotal component of the tissue destruction and disease progression that underlies tumor invasion and arthritis. Agents that target PDPN have shown promising results in preclinical models of arthritis and cancer.

Antibodies that target the extracellular region of PDPN have been shown to inhibit tumor cell migration and metastasis in animal models of cancers, including mesothelioma, glioma, melanoma, and squamous cell carcinoma [84,130–133]. These agents can induce caspase-independent nonapoptotic necrosis of cancer cells, including oral squamous cell carcinoma cells that are resistant to currently available cytotoxic cancer drugs [75,84]. Chimeric antigen receptor transduced T (CAR-T) cells that target PDPN have also been shown to inhibit glioblastoma progression in animal models [134].

Maackia amurensis seed lectin (MASL) has also been found to target α2-3 sialic acid residues on PDPN to inhibit melanoma and oral squamous cell carcinoma growth and metastasis in preclinical models [75,84]. In addition, studies indicate that MASL also targets PDPN on articular chondrocytes to inhibit the production of MMPs, inflammatory cytokines, and arthritic cartilage destruction [111]. MASL is resistant to gastrointestinal digestion, allowing oral administration [135,136]. Moreover, MASL does not produce notable toxicities [75,84,111], and has a long history of use as a 'coincidental component' in traditional medicines used to treat cancer and arthritis [75].

In addition to targeting the extracellular region, PDPN intracellular serines can be phosphorylated by PKA and CDK5 to inhibit cell migration [105,137], and reagents that activate PKA and CDK5 activity can be used to control PDPN-mediated cell migration [137]. Compounds such as CARP1 functional mimetics and disulfiram induce PDPN phosphorylation and inhibit tumor cell migration and invasion [138,139].

Concluding remarks

The Src kinase and PDPN activate destructive pathways that lead to inflammation, cell migration, tissue invasion, and matrix degradation common to both cancer and arthritis. These pathways can be blocked at multiple levels by small molecules and antibodies (Fig. 4). Src kinase activity is widespread and involved in an array of processes. These ubiquitous and pleiotropic effects saddle Src blockers with many adverse effects that render them problematic for therapeutic use. Src effectors present more targeted approaches for pharmaceutical potential. Indeed, PDPN has emerged as a biomarker and therapeutic target in cancer and arthritis. Taken together, surmounting data indicate that agents that target PDPN could be useful to combat both cancer and arthritis.

Conflict of interest

G.S.G. has intellectual property and ownership in Sentrimed, Inc. which is developing agents that target PDPN to treat diseases, including cancer and arthritis.

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References

1 Torre, L.A. *et al.* (2015) Global cancer statistics 2012. *CA Cancer J. Clin.* 65, 87–108
2 Krishnan, H. and Goldberg, G.S. (2015) Contact normalization or escape from the matrix. In *Intercellular Communication in Cancer* (Kandouz, M., ed.), pp. 297–342, Springer
3 Lu, P. *et al.* (2011) Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* 3, a005058
4 Smith, H.A. and Kang, Y. (2013) The metastasis-promoting roles of tumor-associated immune cells. *J. Mol. Med.* 91, 411–429
5 Xing, F. *et al.* (2010) Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Front. Biosci.* 15, 166–179
6 van Kempen, L.C. *et al.* (2006) Inflammation, proteases and cancer. *Eur. J. Cancer* 42, 728–734
7 Schafer, M. and Werner, S. (2008) Cancer as an overhealing wound: an old hypothesis revisited. *Nat. Rev. Mol. Cell Biol.* 9, 628–638
8 Arnold, K.M. *et al.* (2015) Wound healing and cancer stem cells: inflammation as a driver of treatment resistance in breast cancer. *Cancer Growth Metastasis* 8, 1–13
9 Balkwill, F. and Mantovani, A. (2001) Inflammation and cancer: back to Virchow? *Lancet* 357, 539–545
10 Mort, J.S. and Billington, C.J. (2001) Articular cartilage and changes in arthritis: matrix degradation. *Arthritis Res.* 3, 337–341
11 Nagase, H. and Kashiwagi, M. (2003) Aggrecanases and cartilage matrix degradation. *Arthritis Res. Ther.* 5, 94–103
12 Wolf, A.D. and Pfleger, B. (2010) Burden of major musculoskeletal conditions. Policy and practice. Special theme-bone and joint decade 2000–2010. *Bull. World Health Organ.* 81 (9), 646–656
13 Goldring, S.R. and Goldring, M.B. (2006) Clinical aspects, pathology and pathophysiology of osteoarthritis. *J. Musculoskelet. Neuronal Interact.* 6, 376–378
14 Goldring, S.R. and Goldring, M.B. (2016) Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk. *Nat. Rev. Rheumatol.* 12, 632–644
15 Rindfleisch, J.A. and Muller, D. (2005) Diagnosis and management of rheumatoid arthritis. *Am. Fam. Phys.* 72, 1037–1047
16 Firestein, G.S. (2003) Evolving concepts of rheumatoid arthritis. *Nature* 423, 356–361
17 McInnes, I.B. and Schett, G. (2011) The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365 (23), 2205–2219
18 Bartok, B. and Firestein, G.S. (2010) Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol. Rev.* 233, 233–255
19 Chaudhari, K. *et al.* (2016) Rheumatoid arthritis: current and future trends. *Nat. Rev. Drug Discov.* 15, 305–306
20 Malemud, C.J. (2013) Intracellular signaling pathways in rheumatoid arthritis. *J. Clin. Cell. Immunol.* 4, 160
21 Ruderman, E.M. (2012) Overview of safety of non-biologic and biologic DMARDs. *Rheumatology* 51 (Suppl. 6), vi37–vi43
22 Crofford, L.J. (2013) Use of NSAIDs in treating patients with arthritis. *Arthritis Res. Ther.* 15 (Suppl. 3), S2
23 Horn, S.L. and Fentiman, I.S. (2010) The role of non-steroidal anti-inflammatory drugs in the chemoprevention of breast cancer. *Pharmaceuticals* 3, 1550–1560
24 Williams, C.S. *et al.* (1999) The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* 18, 7908–7916
25 Ziegler, J. (1998) Cancer and arthritis share underlying processes. *J. Natl. Cancer Inst.* 90, 802–803
26 Sundy, J.S. (2001) COX-2 inhibitors in rheumatoid arthritis. *Curr. Rheumatol. Rep.* 3, 86–91
27 Coxib and traditional NSAID Trialists' (CNT) Collaboration *et al.* (2013) Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 382, 769–779
28 Half, E. and Arber, N. (2009) Colon cancer: preventive agents and the present status of chemoprevention. *Expert Opin. Pharmacother.* 10, 211–219
29 North, G.L. (2001) Celecoxib as adjunctive therapy for treatment of colorectal cancer. *Ann. Pharmacother.* 35, 1638–1643
30 Krishnan, H. *et al.* (2012) SRC points the way to biomarkers and chemotherapeutic targets. *Genes Cancer* 3, 426–435
31 Thomas, S.M. and Brugge, J.S. (1997) Cellular functions regulated by Src family kinases. *Annu. Rev. Cell. Dev. Biol.* 13, 513–609
32 Avizienyte, E. and Frame, M.C. (2005) Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. *Curr. Opin. Cell Biol.* 17, 542–547
33 Westhoff, M.A. *et al.* (2004) SRC-mediated phosphorylation of focal adhesion kinase couples actin and adhesion dynamics to survival signaling. *Mol. Cell Biol.* 24, 8113–8133
34 Irby, R.B. and Yeatman, T.J. (2000) Role of Src expression and activation in human cancer. *Oncogene* 19, 5636–5642
35 Summy, J.M. and Gallick, G.E. (2003) Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev.* 22, 337–358
36 Kolli-Bouhafs, K. *et al.* (2014) FAK competes for Src to promote migration against invasion in melanoma cells. *Cell Death Dis.* 5, e1379
37 Mitra, S.K. and Schlaepfer, D.D. (2006) Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol.* 18, 516–523
38 Lauzier, A. *et al.* (2011) Formation of invadopodia-like structures by synovial cells promotes cartilage breakdown in collagen-induced arthritis: involvement of the protein tyrosine kinase Src. *Arthritis Rheum.* 63, 1591–1602
39 Charbonneau, M. *et al.* (2016) Platelet-derived growth factor receptor activation promotes the prodestructive invadosome-forming phenotype of synoviocytes from patients with rheumatoid arthritis. *J. Immunol.* 196, 3264–3275
40 Shahrara, S. *et al.* (2007) Differential expression of the FAK family kinases in rheumatoid arthritis and osteoarthritis synovial tissues. *Arthritis Res. Ther.* 9, R112
41 Okutani, D. *et al.* (2006) Src protein tyrosine kinase family and acute inflammatory responses. *Am. J. Physiol. Lung Cell Mol. Physiol.* 291, L129–L141
42 Lowell, C.A. (2011) Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. *Cold Spring Harb. Perspect. Biol.* 3, a002352
43 Kovacs, M. *et al.* (2014) The Src family kinases Hck, Fgr, and Lyn are critical for the generation of the in vivo inflammatory environment without a direct role in leukocyte recruitment. *J. Exp. Med.* 211, 1993–2011
44 Abu-Amer, Y. *et al.* (1998) Tumor necrosis factor-alpha activation of nuclear transcription factor-kappaB in marrow macrophages is mediated by c-Src tyrosine phosphorylation of Ikappa Balpha. *J. Biol. Chem.* 273, 29417–29423
45 Parameswaran, N. and Patial, S. (2010) Tumor necrosis factor-alpha signaling in macrophages. *Crit. Rev. Eukaryot. Gene Expr.* 20, 87–103
46 Lee, A.W. and States, D.J. (2000) Both Src-dependent and -independent mechanisms mediate phosphatidylinositol 3-kinase regulation of colony-stimulating factor 1-activated mitogen-activated protein kinases in myeloid progenitors. *Mol. Cell. Biol.* 20, 6779–6798
47 Morel, J.C. *et al.* (2002) Signal transduction pathways involved in rheumatoid arthritis synovial fibroblast interleukin-18-induced vascular cell adhesion molecule-1 expression. *J. Biol. Chem.* 277, 34679–34691
48 Mor, A. *et al.* (2005) The fibroblast-like synovial cell in rheumatoid arthritis: a key player in inflammation and joint destruction. *Clin. Immunol.* 115, 118–128
49 Wada, T. *et al.* (2006) RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Mol. Med.* 12, 17–25
50 Jones, D.H. *et al.* (2002) Role of RANKL and RANK in bone loss and arthritis. *Ann. Rheum. Dis.* 61 (Suppl. 2), ii32–ii39
51 Byeon, S.E. *et al.* (2012) The role of Src kinase in macrophage-mediated inflammatory responses. *Mediators Inflamm.* 2012, 512926
52 Bursell, L. *et al.* (2007) Src kinase inhibition promotes the chondrocyte phenotype. *Arthritis Res. Ther.* 9, R105
53 D'Aura Swanson, C. *et al.* (2009) Tyrosine kinases as targets for the treatment of rheumatoid arthritis. *Nat. Rev. Rheumatol.* 5, 317–324
54 Zhang, S. and Yu, D. (2012) Targeting Src family kinases in anti-cancer therapies: turning promise into triumph. *Trends Pharmacol. Sci.* 33, 122–128
55 Kim, L.C. *et al.* (2009) Src kinases as therapeutic targets for cancer. *Nat. Rev. Clin. Oncol.* 6, 587–595
56 Aleem, S. *et al.* (2016) Structural and biochemical basis for intracellular kinase inhibition by Src-specific peptidic macrocycles. *Cell. Chem. Biol.* 23, 1103–1112
57 Gomez-Puerta, J.A. and Mocsai, A. (2013) Tyrosine kinase inhibitors for the treatment of rheumatoid arthritis. *Curr. Top. Med. Chem.* 13, 760–773

58 Elias, D. and Ditzel, H.J. (2015) The potential of Src inhibitors. *Aging* 7, 734–735
59 Lodish, M.B. (2013) Clinical review: kinase inhibitors: adverse effects related to the endocrine system. *J. Clin. Endocrinol. Metab.* 98, 1333–1342
60 Shen, Y. *et al.* (2010) SRC induces podoplanin expression to promote cell migration. *J. Biol. Chem.* 285, 9649–9656
61 Retzbach, E.P. *et al.* (2018) Podoplanin emerges as a functionally relevant oral cancer biomarker and therapeutic target. *Oral Oncol.* 78, 126–136
62 Scholl, F.G. *et al.* (1999) Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. *J. Cell Sci.* 112, 4601–4613
63 Martin-Villar, E. *et al.* (2006) Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. *J. Cell Sci.* 119, 4541–4553
64 Wicki, A. *et al.* (2006) Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell* 9, 261–272
65 Inoue, H. *et al.* (2012) Podoplanin promotes cell migration via the EGF-Src-Cas pathway in oral squamous cell carcinoma cell lines. *J. Oral Sci.* 54, 241–250
66 Rubin, H. (2008) Contact interactions between cells that suppress neoplastic development: can they also explain metastatic dormancy? *Adv. Cancer Res.* 100, 159–202
67 Martin-Villar, E. *et al.* (2009) Regulation of podoplanin/PA2.26 antigen expression in tumour cells. Involvement of calpain-mediated proteolysis. *Int. J. Biochem. Cell. Biol.* 41, 1421–1429
68 Grzegrzolka, J. *et al.* (2017) Correlation between expression of twist and podoplanin in ductal breast carcinoma. *Anticancer Res.* 37, 5485–5493
69 Ernst, A. *et al.* (2009) Genomic and expression profiling of glioblastoma stem cell-like spheroid cultures identifies novel tumor-relevant genes associated with survival. *Clin. Cancer Res.* 15, 6541–6550
70 Riedl, J. *et al.* (2017) Podoplanin expression in primary brain tumors induces platelet aggregation and increases risk of venous thromboembolism. *Blood* 129, 1831–1839
71 Kolar, K. *et al.* (2015) Podoplanin: a marker for reactive gliosis in gliomas and brain injury. *J. Neuropathol. Exp. Neurol.* 74, 64–74
72 Shibahara, J. *et al.* (2006) Podoplanin is expressed in subsets of tumors of the central nervous system. *Virchows Arch.* 448, 493–499
73 Watanabe, M. *et al.* (1990) Expression of a Mr 41,000 glycoprotein associated with thrombin-independent platelet aggregation in high metastatic variants of murine B16 melanoma. *Cancer Res.* 50, 6657–6662
74 Monzani, E. *et al.* (2007) Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur. J. Cancer* 43, 935–946
75 Ochoa-Alvarez, J.A. *et al.* (2012) Plant lectin can target receptors containing sialic acid, exemplified by podoplanin, to inhibit transformed cell growth and migration. *PLoS One* 7, e41845
76 Kan, S. *et al.* (2014) Podoplanin expression in cancer-associated fibroblasts predicts aggressive behavior in melanoma. *J. Cutan. Pathol.* 41, 561–567
77 Ogasawara, S. *et al.* (2016) Podoplanin expression in canine melanoma. *Monoclon Antibodies Immunodiagn. Immunother.* 35, 304–306
78 Kimura, N. and Kimura, I. (2005) Podoplanin as a marker for mesothelioma. *Pathol. Int.* 55, 83–86
79 Ordonez, N.G. (2005) D2-40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. *Hum. Pathol.* 36, 372–380
80 Takeuchi, S. *et al.* (2017) Podoplanin promotes progression of malignant pleural mesothelioma by regulating motility and focus formation. *Cancer Sci.* 108, 696–703
81 Martin-Villar, E. *et al.* (2005) Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. *Int. J. Cancer* 113, 899–910
82 Kreppel, M. *et al.* (2010) Impact of podoplanin expression in oral squamous cell carcinoma: clinical and histopathologic correlations. *Virchows Arch.* 456, 473–482
83 Swain, N. *et al.* (2014) Podoplanin—a novel marker in oral carcinogenesis. *Tumour Biol.* 35, 8407–8413
84 Ochoa-Alvarez, J.A. *et al.* (2015) Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. *Oncotarget* 6, 9045–9060
85 Wicki, A. and Christofori, G. (2007) The potential role of podoplanin in tumour invasion. *Br. J. Cancer* 96, 1–5
86 Raica, M. *et al.* (2008) The role of podoplanin in tumor progression and metastasis. *Anticancer Res.* 28 (5B), 2997–3006
87 Ordonez, N.G. (2014) Value of podoplanin as an immunohistochemical marker in tumor diagnosis: a review and update. *Appl. Immunohistochem. Mol. Morphol.* 22, 331–347
88 Renart, J. *et al.* (2015) New insights into the role of podoplanin in epithelial-mesenchymal transition. *Int. Rev. Cell. Mol. Biol.* 317, 185–239
89 Krishnan, H. *et al.* (2018) Podoplanin: an emerging cancer biomarker and therapeutic target. *Cancer Sci.* 109, 1292–1299
90 Hwang, Y.S. *et al.* (2012) Functional invadopodia formation through stabilization of the PDPN transcript by IMP-3 and cancer-stromal crosstalk for PDPN expression. *Carcinogenesis* 33, 2135–2146
91 Martin-Villar, E. *et al.* (2015) Podoplanin mediates ECM degradation by squamous carcinoma cells through control of invadopodia stability. *Oncogene* 34, 4531–4544
92 Li, Y.Y. *et al.* (2015) Podoplanin promotes the invasion of oral squamous cell carcinoma in coordination with MT1-MMP and Rho GTPases. *Am. J. Cancer Res.* 5, 514–529
93 Monteiro, L.S. *et al.* (2016) Prognostic significance of CD44v6, p63, podoplanin and MMP-9 in oral squamous cell carcinomas. *Oral Dis.* 22, 303–312
94 Li, Y.Y. *et al.* (2018) Interaction between oral squamous cell carcinoma cells and fibroblasts through TGF-beta1 mediated by podoplanin. *Exp. Cell Res.* 369, 43–53
95 Maruyama, S. *et al.* (2018) Podoplanin expression as a prognostic factor in gastric cancer. *Anticancer Res.* 38, 2717–2722
96 Kitano, H. *et al.* (2010) Podoplanin expression in cancerous stroma induces lymphangiogenesis and predicts lymphatic spread and patient survival. *Arch. Pathol. Lab. Med.* 134, 1520–1527
97 Inoue, H. *et al.* (2014) Podoplanin expressing cancer-associated fibroblasts in oral cancer. *Tumour Biol.* 35, 11345–11352
98 Pula, B. *et al.* (2011) Podoplanin expression by cancer-associated fibroblasts predicts poor outcome in invasive ductal breast carcinoma. *Histopathology* 59, 1249–1260
99 Schoppmann, S.F. *et al.* (2012) Podoplanin-expressing cancer-associated fibroblasts are associated with poor prognosis in invasive breast cancer. *Breast Cancer Res. Treat.* 134, 237–244
100 Shindo, K. *et al.* (2013) Podoplanin expression in cancer-associated fibroblasts enhances tumor progression of invasive ductal carcinoma of the pancreas. *Mol. Cancer* 12, 168
101 Astarita, J.L. *et al.* (2012) Podoplanin: emerging functions in development, the immune system, and cancer. *Front. Immunol.* 3, 283
102 Pula, B. *et al.* (2013) Significance of podoplanin expression in cancer-associated fibroblasts: a comprehensive review. *Int. J. Oncol.* 42, 1849–1857
103 Kawase, A. *et al.* (2008) Podoplanin expression by cancer associated fibroblasts predicts poor prognosis of lung adenocarcinoma. *Int. J. Cancer* 123, 1053–1059
104 Schoppmann, S.F. *et al.* (2013) Podoplanin expressing cancer associated fibroblasts are associated with unfavourable prognosis in adenocarcinoma of the esophagus. *Clin. Exp. Metastasis* 30, 441–446
105 Krishnan, H. *et al.* (2013) Serines in the intracellular tail of podoplanin (PDPN) regulate cell motility. *J. Biol. Chem.* 288, 12215–12221
106 Ekwall, A.K. *et al.* (2011) The tumour-associated glycoprotein podoplanin is expressed in fibroblast-like synoviocytes of the hyperplastic synovial lining layer in rheumatoid arthritis. *Arthritis Res. Ther.* 13, R40
107 Miyamoto, Y. *et al.* (2013) Podoplanin is an inflammatory protein upregulated in Th17 cells in SKG arthritic joints. *Mol. Immunol.* 54, 199–207
108 Del Rey, M.J. *et al.* (2014) Clinicopathological correlations of podoplanin (gp38) expression in rheumatoid synovium and its potential contribution to fibroblast platelet crosstalk. *PLoS One* 9, e99607
109 Talmon, G. *et al.* (2013) Podoplanin and clusterin are reliable markers of nonneoplastic synovium at various sites. *Int. J. Surg. Pathol.* 21, 587–590
110 Takakubo, Y. *et al.* (2017) Distribution of podoplanin in synovial tissues in rheumatoid arthritis patients using biologic or conventional disease-modifying anti-rheumatic drugs. *Curr. Rheumatol. Rev.* 13, 72–78
111 Mayan-Santos, M.D. *et al. Compositions and methods to treat inflammatory joint disease*. Rowan University.. PCT/US14/ 45229
112 Choi, I.Y. *et al.* (2017) Stromal cell markers are differentially expressed in the synovial tissue of patients with early arthritis. *PLoS One* 12, e0182751
113 Lowe, K.L. *et al.* (2012) Platelet CLEC-2 and podoplanin in cancer metastasis. *Thromb. Res.* 129 (Suppl. 1), S30–37
114 Miyata, K. *et al.* (2017) Podoplanin enhances lung cancer cell growth in vivo by inducing platelet aggregation. *Sci. Rep.* 7, 4059
115 Takemoto, A. *et al.* (2017) A critical role of platelet TGF-beta release in podoplanin—mediated tumour invasion and metastasis. *Sci. Rep.* 7, 42186
116 Kunita, A. *et al.* (2007) The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. *Am. J. Pathol.* 170, 1337–1347
117 Chang, Y.W. *et al.* (2015) Identification of a novel platelet antagonist that binds to CLEC-2 and suppresses podoplanin-induced platelet aggregation and cancer metastasis. *Oncotarget* 6, 42733–42748
118 Sasaki, T. *et al.* (2018) Functional characterization of recombinant snake venom rhodocytin: rhodocytin mutant blocks CLEC-2/podoplanin-dependent platelet aggregation and lung metastasis. *J. Thromb. Haemost.* 16, 960–972

119 Gitz, E. *et al.* (2014) CLEC-2 expression is maintained on activated platelets and on platelet microparticles. *Blood* 124, 2262–2270
120 Boilard, E. *et al.* (2010) Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 327, 580–583
121 Rayes, J. *et al.* (2017) The podoplanin-CLEC–2 axis inhibits inflammation in sepsis. *Nat. Commun.* 8, 2239
122 Kunita, A. *et al.* (2018) Inflammatory cytokines induce podoplanin expression at the tumor invasive front. *Am. J. Pathol.* 188, 1276–1288
123 Noack, M. *et al.* (2016) Interaction among activated lymphocytes and mesenchymal cells through podoplanin is critical for a high IL–17 secretion. *Arthritis Res. Ther.* 18, 148
124 Gresele, P. *et al.* (2017) Matrix metalloproteinases and platelet function. *Prog. Mol. Biol. Transl. Sci.* 147, 133–165
125 Nurden, A.T. (2011) Platelets, inflammation and tissue regeneration. *Thromb. Haemost.* 105 (Suppl. 1), S13–S33
126 Jurasz, P. *et al.* (2003) Matrix metalloproteinase-2 contributes to increased platelet reactivity in patients with metastatic prostate cancer: a preliminary study. *Thromb. Res.* 112, 59–64
127 Alunno, A. *et al.* (2017) Platelets contribute to the accumulation of matrix metalloproteinase type 2 in synovial fluid in osteoarthritis. *Thromb. Haemost.* 117, 2116–2124
128 Elliott, S. and Cawston, T. (2001) The clinical potential of matrix metalloproteinase inhibitors in the rheumatic disorders. *Drugs Aging* 18, 87–99
129 Cathcart, J. *et al.* (2015) Targeting matrix metalloproteinases in cancer: bringing new life to old ideas. *Genes Dis.* 2, 26–34
130 Kato, Y. and Kaneko, M.K. (2014) A cancer-specific monoclonal antibody recognizes the aberrantly glycosylated podoplanin. *Sci. Rep.* 4, 5924
131 Kato, Y. *et al.* (2015) The chimeric antibody chLpMab-7 targeting human podoplanin suppresses pulmonary metastasis via ADCC and CDC rather than via its neutralizing activity. *Oncotarget* 6, 36003–36018
132 Sekiguchi, T. *et al.* (2016) Targeting a novel domain in podoplanin for inhibiting platelet-mediated tumor metastasis. *Oncotarget* 7, 3934–3946
133 Ogasawara, S. *et al.* (2016) Establishment of mouse monoclonal antibody LpMab-13 against human podoplanin. *Monoclon Antibodies Immunodiagn. Immunother.* 35, 155–162
134 Shiina, S. *et al.* (2016) CAR T cells targeting podoplanin reduce orthotopic glioblastomas in mouse brains. *Cancer Immunol. Res.* 4, 259–268
135 Liu, B. *et al.* (2010) Plant lectins: potential antineoplastic drugs from bench to clinic. *Cancer Lett.* 287, 1–12
136 Wang, Q. *et al.* (1998) Identification of intact peanut lectin in peripheral venous blood. *Lancet* 352, 1831–1832
137 Krishnan, H. *et al.* (2015) PKA and CDK5 can phosphorylate specific serines on the intracellular domain of podoplanin (PDPN) to inhibit cell motility. *Exp. Cell. Res.* 335, 115–122
138 Ashour, A.E. *et al.* (2013) CARP-1 functional mimetics: a novel class of small molecule inhibitors of medulloblastoma cell growth. *PLoS One* 8, e66733
139 Cheriyan, V.T. *et al.* (2014) Disulfiram suppresses growth of the malignant pleural mesothelioma cells in part by inducing apoptosis. *PLoS One* 9, e93711